Linzhao Cheng

Application No.: 10/533,514 Filed: January 23, 2006

Page 2

## **Amendments to the Claims:**

Please amend claims 1, 10, 18, and 52, as provided below in the Listing of Claims.

This Listing of Claims will replace all prior version, and listings, of claims in the application:

## **Listing of Claims:**

- 1. (Currently Amended) Isolated undifferentiated pluripotential human embryonic stem (hES) cells, wherein the hES cells are passage 4 or higher, have a diploid karyotype, and exhibit dependence on adult human feeder cells or an hES cell-maintaining product of the adult human feeder cells[[,]] for maintenance in culture in an undifferentiated state for 4 or more passages, wherein the adult human feeder cells comprise human bone marrow cells or human fibroblasts from breast skin.
- 2. (Original) The hES cells of claim 1, wherein the adult human feeder cells comprise human bone marrow cells.
- 3. (Original) The hES cells of claim 2, wherein the human bone marrow cells comprise human marrow stromal cells.
- 4. (Original) The hES cells of claim 1, wherein the adult human feeder cells comprise human fibroblasts.
- 5. (Original) The hES cells of claim 4, wherein the human fibroblasts comprise ATCC CCD-1087sk cells.
- 6. (Original) The hES cells of claim 1, wherein the adult human feeder cells comprise immortalized cells.

Linzhao Cheng Application No.: 10/533,514

Filed: January 23, 2006 Page 3

7. (Previously Presented) The hES cells of claim 6, wherein the immortalized cells contain an exogenous polynucleotide encoding a telomerase, which is expressed in the immortalized cells.

- 8. (Previously Presented) The hES cells of claim 1, wherein the product of the adult human feeder cells comprises conditioned medium.
- 9. (Previously Presented) The hES cells of claim 1, wherein the product of the adult human feeder cells comprises a fraction of conditioned medium comprising biomolecules having a molecular mass greater than about 30 kiloDaltons.
- 10. (Currently Amended) A culture of undifferentiated pluripotential human embryonic stem (hES) cells, comprising:

hES cells, wherein the hES cells are passage 4 or higher and have a diploid karyotype, and

supportive adult human feeder cells, or an hES cell-maintaining product of the adult human feeder cells, wherein the adult human feeder cells comprise human bone marrow cells or human fibroblasts from breast skin.

- 11. (Original) The culture of claim 10, comprising the hES cells and the supportive adult human feeder cells.
- 12. (Original) The culture of claim 10, comprising the hES cells and the hES cellmaintaining product of the supportive adult human feeder cells.
  - 13. (Original) The culture of claim 12, further comprising non-supportive feeder cells.
- 14. (Original) The culture of claim 13, wherein the non-supportive feeder cells are human cells.

Linzhao Cheng Application No.: 10/533,514

Filed: January 23, 2006

Page 4

15. (Original) The culture of claim 10, wherein the supportive adult human feeder cells comprise human bone marrow stromal cells or ATCC CCD-1087sk fibroblasts.

16. (Original) The culture of claim 10, wherein the hES cell-maintaining product of the supportive adult human feeder cells comprises conditioned medium, or a fraction of conditioned medium comprising biomolecules having a molecular mass greater than about 30 kiloDaltons.

17. (Previously Presented) A method of obtaining an expanded population of undifferentiated pluripotential human embryonic stem (hES) cells, comprising culturing hES cells, and supportive adult human feeder cells or an hES cell-maintaining product of the feeder cells, wherein the adult human feeder cells comprise human fibroblasts from breast skin, under conditions suitable for growth of the hES cells, thereby obtaining an expanded population of the hES cells.

- 18. (Currently Amended) An expanded culture of undifferentiated pluripotential hES cells prepared by the method of claim 17.
- 19. (Original) The method of claim 17, comprising culturing the hES cells and the supportive adult human feeder cells.
- 20. (Previously Presented) The method of claim 17, wherein the supportive adult human feeder cells comprise ATCC CCD-1087sk fibroblasts or a combination of human bone marrow stromal cells and ATCC CCD-1087sk fibroblasts.
- 21. (Original) The method of claim 17, wherein the supportive adult human feeder cells are immortalized.

Application No.: 10/533,514

Filed: January 23, 2006

Page 5

- 22. (Original) The method of claim 17, comprising culturing the hES cells and the hES cell-maintaining product of the supportive adult human feeder cells.
- 23. (Previously Presented) The method of claim 22, further comprising culturing the hES cells and the supportive adult human feeder cells, or the hES cell-maintaining product of the feeder cells, with non-supportive feeder cells.
- 24. (Original) The method of claim 17, further comprising isolating hES cells of the expanded population of hES cells, thereby obtaining isolated undifferentiated pluripotential hES cells.
- 25. (Original) Isolated undifferentiated pluripotential hES cells obtained by the method of claim 24.
- 26. (Original) The method of claim 17, further comprising sub-culturing hES cells of the expanded population of hES cells under conditions suitable for growth, thereby obtaining a sub-culture of hES cells.
- 27. (Previously Presented) The method of claim 26, comprising sub-culturing the hES cells of the expanded population, and supportive adult human feeder cells or an hES cellmaintaining product of the feeder cells, under conditions suitable for growth of the hES cells.
- 28. (Original) The method of claim 26, further comprising repeating the sub-culturing step, thereby obtaining a continuous culture of undifferentiated pluripotential hES cells.
- 29. (Original) The method of claim 27, further comprising, before sub-culturing the hES cells, freezing an aliquot of the expanded population of hES cells.

Application No.: 10/533,514 Filed: January 23, 2006

Page 6

30. (Original) The method of claim 29, wherein freezing the cells is performed under conditions such that the cells remain viable.

Claims 31-33. (Canceled)

- 34. (Withdrawn) The method of claim 17, further comprising inducing differentiation of hES cells of the expanded population, thereby obtaining a population of differentiated cells.
- 35. (Withdrawn) A population of differentiated cells obtained by the method of claim 34.
- 36. (Withdrawn) A method for identifying an agent that alters a function of an undifferentiated pluripotential human embryonic stem (hES) cell, comprising:
  - a) contacting the hES cells with a test agent,

wherein the hES cells exhibit dependence on adult human feeder cells, or an hES cell-maintaining product of said adult human feeder cells, for maintenance in culture; and

- b) detecting a change in a function of the hES cells in presence of the test agent as compared to the function in the absence of the test agent, thereby identifying the test agent as an agent that alters the function of the hES cells.
- 37. (Withdrawn) The method of claim 36, wherein said contacting is performed in vivo.
- 38. (Withdrawn) The method of claim 36, wherein said contacting is performed in vitro.
- 39. (Withdrawn) The method of claim 36, wherein the function of the hES cells is expression of stage-specific surface antigen-4 (SSEA-4), alkaline phosphatase, or Oct-4 transcription factor.

Application No.: 10/533,514 Filed: January 23, 2006

Page 7

- 40. (Withdrawn) The method of claim 36, wherein the agent induces differentiation of the hES cells, thereby producing differentiated cells.
- 41. (Withdrawn) The method of claim 40, wherein the differentiated cells comprise multipotential human stem cells.
- 42. (Withdrawn) The method of claim 41, wherein the multipotential human stem cells comprise hematopoietic stem cells.
- 43. (Withdrawn) The method of claim 40, wherein the differentiated cells comprise terminally differentiated cells.
- 44. (Withdrawn) The method of claim 40, wherein the differentiated cells comprises muscle cells, neuronal cells, blood cells, connective tissue, or epithelial cells.
- 45. (Withdrawn) The method of claim 40, wherein the differentiated cells comprise pancreatic beta cells, hepatocytes, cardiomyocytes, or skeletal muscle cells.
- 46. (Withdrawn) A method of obtaining a cell culture medium for maintaining undifferentiated pluripotential human embryonic stem (hES) cell in culture, comprising:
  - a) culturing adult human cells that can support the growth of hES cells in culture; and
  - b) isolating conditioned medium generated by culturing the adult human cells, thereby obtaining a cell culture medium for maintaining undifferentiated pluripotential hES cells in culture.
- 47. (Withdrawn) The method of claim 46, wherein the adult human cells comprise human bone marrow stromal cells or ATCC CCD-1087sk fibroblasts.

In re Application of
Linzhao Cheng

Attorney Docket No. JHU1910-5

Application No.: 10/533,514 Filed: January 23, 2006

Page 8

48. (Withdrawn) The method of claim 46, further comprising isolating from the conditioned medium a fraction comprising biomolecules having a molecular mass greater than about 30 kiloDaltons.

- 49. (Withdrawn) The method of claim 48, wherein isolating the fraction comprises collecting a gel chromatography fraction.
  - 50. (Withdrawn) Conditioned medium obtained by the method of claim 46.
- 51. (Withdrawn) Enriched hES cell growth factors, comprising a fraction of the conditioned medium of claim 50 comprising biomolecules having a molecular mass greater than about 30 kiloDaltons.
- 52. (Currently Amended) A method for obtaining undifferentiated pluripotential human embryonic stem (hES) cells, comprising:
  - a) culturing a suspension of cells comprising undifferentiated pluripotential hES cells, and supportive adult human feeder cells or an hES cell-maintaining product of the feeder cells, under conditions suitable for growth of the hES cells, wherein the adult human feeder cells comprise human fibroblasts from breast skin; and
  - b) isolating cells that express stage-specific surface antigen-4 (SSEA-4), Oct-4, and alkaline phosphatase, and do not express stage-specific surface antigen-1 (SSEA-1), thereby obtaining undifferentiated pluripotential hES cells.
- 53. (Previously Presented) The method of claim 52, wherein the suspension of cells comprises the hES cells and the supportive adult human feeder cells.
- 54. (Previously Presented) The method of claim 52, wherein the suspension of cells comprises the hES cells and the hES cell-maintaining product of the supportive adult human feeder cells.

Linzhao Cheng Application No.: 10/533,514

Filed: January 23, 2006

Page 9

55. (Previously Presented) The method of claim 54, wherein the suspension of cells further comprises non-supportive feeder cells.

56. (Original) Isolated undifferentiated pluripotential hES cells obtained by the method of claim 52.

57. (Withdrawn) A method of ameliorating a pathologic condition in a subject, comprising administering undifferentiated pluripotential human embryonic stem (hES) cells, or cells derived from said hES cells, to the subject,

wherein the hES cells exhibit dependence on adult human feeder cells, or an hES cell-maintaining product of said adult human feeder cells, for maintenance in culture.

58. (Withdrawn) The method of claim 57, wherein the pathologic condition comprises a degenerative disorder.

59. (Withdrawn) The method of claim 58, wherein the degenerative disorder comprises Parkinson's disease, Alzheimer's disease, or muscular dystrophy.

60. (Withdrawn) The method of claim 57, wherein the pathologic condition comprises an autoimmune disorder.

61. (Withdrawn) The method of claim 60, wherein the autoimmune disorder is multiple sclerosis.

62. (Withdrawn) The method of claim 57, wherein the pathologic condition is diabetes.

63. (Withdrawn) The method of claim 57, wherein the pathologic condition comprises an injury.

In re Application of Linzhao Cheng

PATENT Attorney Docket No. JHU1910-5

Application No.: 10/533,514 Filed: January 23, 2006

Page 10

64. (Withdrawn) The method of claim 63, wherein the injury comprises a spinal cord injury or a burn.